BOTANICAL GAZETTE

DECEMBER, 1893.

The bacterial flora of the Atlantic ocean in the vicinity of Woods Holl, Mass.

A contribution to the morphology and physiology of marine bacteria.

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WITH PLATE XXXVI.

(Concluded from p. 417.)

Description of new forms.

While some of the forms that have been isolated at Woods Holl have been identified with those found in Mediterranean waters, the predominant species have in most cases specific differences that separate them from the European species. A careful search in the systematic works of Eisenberg, Frankland, Lustig, and others that describe mainly the water forms failed to give any evidence that the forms here dealt with had been previously recorded. ' This is not at all surprising when we take into consideration the fact that the systematic part of bacteriology has heretofore concerned itself mainly with the fresh water types and that little or no attention has been given the marine bacterial flora.

I have, therefore, described the predominating species that have been isolated and studied during the past season in order that we may have a basis for comparison with European marine forms.

The difficulties of a satisfactory classification of bacterial species have not as yet been entirely overcome, but the present tendency to introduce physiological and ecological characters in the description of new forms is virtually a necessity. The correlation of form and function in the living organism is so close that both factors must be considered in any accurate and thorough description that will enable a later student to positively identify the species.

The bacterial flora of any particular habitat may be considered in two ways—qualitatively or quantitatively. Often there is a wide diversity as to the number of different forms that are present in any particular location, but as a rule the majority of individual organisms belong within the limits of comparatively few species. This is more especially true with reference to a region in which the adventive or introduced forms are comparatively few, and with the great bulk of oceanic waters these conditions prevail. Where they receive the drainage of large river systems or are subject to sewage contamination of large cities, the number of species as well as the number of individual germs is usually increased, but these conditions do not obtain with reference to the locality investigated this season.

The location of Woods Holl is so favorable with respect to the possibility of the introduction of adventive forms that it seems highly probable that the conditions here are quite normal and may fairly represent the condition of marine bacterial life in general.

While the water and mud masses of this region possess bacterial life in considerable abundance, no great variety of species is to be noted. The four or five forms that will be described in detail comprise by far the larger proportion of living germs that are to be found. In numerous instances, the cultures contained not more than two or three species, and very rarely did any single culture show more than four or five different forms.

This peculiarity is quite noteworthy, as the number of predominating species of any particular habitat is rarely so small as in this case.

Bacillus limicola, sp. nov.—This species is the most characteristic indigenous inhabitant of the ocean bottom in the Atlantic near Woods Holl. Almost every test that was made from the mud revealed the presence of this bacillus.

In regard to its morphological characters, it is a bacillus, but it has a variability of form and size that is quite remarkable. If a cover glass preparation be made from a rapidly developing agar culture, such wide diversity of forms present themselves, that one can scarcely avoid the conclusion that the culture has become contaminated, even though he may be satisfied as to the purity of its origin. There are bunches of short oval forms, almost spherical, that closely resemble large micrococci (fig. 1, *B*, *a*), others of an elongated normal bacillus type, often united in short filaments (fig. 1, *B*, *c*), while still others are curved after the manner of a spirillum.

The variability of form is also accompanied by a wide range in size, not only among the different growth forms but among those of the same morphological structure. Besides what seems to be the typical, elongated bacillus, there are often seen individual cells, having one end pointed, or lance-shaped (fig. 1, *B*, *b*). These cells are usually united in pairs, the larger ends being in contact and present a strong resemblance to the lanceolated forms found in Bac. pneumoniæ. The range in size of the bacillus type of this germ varies within the limits of $0.7-1.8\mu$ in width, while the length is sometimes no more than 1μ and from that to 5μ. The short spheroidal cells are as a rule somewhat wider than the bacillus type, and have a more refringent aspect that closely recalls the appearance of arthrospores of some species, but the cell union of these forms would hardly be conceivable with true spores.

Then, too, the fact that old non-sporogenous cultures, having these refringent cells, were killed at the comparatively low temperature of 55°C. is against the view that they are genuine spore stages of this bacillus.

Whether the wide variation in form and size is due to the pleomorphic nature of the species or to involution changes is a question. These appearances are to be noted in cultures 24hrs old and in hanging drop preparations the unstained bacteria have the ordinary optical properties of active protoplasm, but when subjected to staining agents like methylene blue or carbol-fuchsin, they stain so irregularly as to lead one to believe that a degeneration of the protoplasm has already begun. Cover-glass preparations stained by Gram's method are not decolorized.

When the germ was first isolated at Woods Holl, spores were often observed in agar cultures kept at ordinary summer heat, but in later cultivations on both salt and fresh water media, I have been unable to demonstrate them either at room temperature or blood heat. This peculiarity is somewhat remarkable, yet the observance of this phenomenon with other bacterial forms like anthrax when cultivated on artificial media for considerable time shows that this condition is not exceptional. It is another instance that goes to show how intimate the relation is between the organism and its environment; how that by modification of exterior surroundings, such a deep seated phenomenon as that of reproduction may be profoundly affected.

The chromogenic function of this bacillus is best seen when grown on an agar medium. On this substratum, it presents a fairly copious growth, smooth and shining. At first the color is but slightly developed, but as the culture increases in age, it passes from an ochreous yellow to a deep rich orange. There is considerable variation in the production of the pigment depending upon the temperature at which the culture is grown. Cultures kept at blood heat remain very pale and do not develop, even in old age, the rich orange tint that is seen in growths kept at the temperature of the room.

In gelatin the growth characters are as follows: In tube cultures 24 hours old, there is a slight pit observable at surface point of inoculation caused by the liquefaction of the gelatin. This spreads superficially, and also gradually deepens until there is a broad liquefied funnel formed in the gelatin that slowly widens until the sides of the tube are reached. Usually there persists for several days at the surface, an air pit in the medium, caused by the evaporation of liquefied material. The consistency of this liquid mass is thick but not viscous. The fluid gelatin is filled with a slightly flocculent precipitate that remains in suspension, while a considerable amount of amorphous yellowish growth material is deposited on bottom. No membrane is formed on the surface of the fluid.

The appearance of colonies in gelatin plates is not especially characteristic. The germs quickly liquefy the medium in a regular circular form. Macroscopically, the edge of the colony is translucent while opaque patches of zooglœa cover the center. These are usually of a dull, grey color, but are sometimes tinged with a faint reddish lustre when observed in reflected light. Under the lens, the edge of the colony is granular, the extreme periphery being the densest. Short irregular filaments radiate from the extreme edge into the surrounding gelatin.

On agar, the germ develops quite rapidly although not very luxuriantly. The culture appears on the second day as a smooth, shining colony that spreads out in a thin layer over surface of medium.

The young cultures are often tinged with yellow, but this gradually deepens with age until it reaches a rich orange brown.

On potato, no growth could be obtained, while in milk, the casein was entirely peptonized although but a slight amount of acid was produced. The milk serum remains turbid. Under anaerobic culture conditions, no growth could be noted.

Bacillus pelagicus, sp. nov.—One of the most common and widely distributed of the marine bacteria that have been observed in this locality is a small, short bacillus that has been named B. pelagicus.

This species was found abundantly in both water and mud, and in almost every culture that was made. The mud layers were filled with it, both in a vegetative and a spore condition, so that it is evident that its presence on the sea bottom is not to be accounted for by simple deposition of the spores from the water masses above. It is found abundantly in the vicinity of the shore, as well as in the samples that were taken by the *Grampus* 100 miles from land.

Morphologically, it is a slender bacillus, usually quite short ($0.4\mu \times 1.5$–2.5μ), but a number of segments may be united into filaments of various lengths. In vigorous cultures, the shorter forms have a very lively, tumbling movement. Small, narrow spores are easily formed on different media at ordinary temperatures. This bacillus takes aniline stains easily. When Gram's solution is used, the bacilli show a spotted appearance, the granules retaining the stain while the remainder of the cell is decolorized.

In cultures it grows fairly well, although not luxuriantly. In gelatin plate cultures growth is not rapid, but the food-medium is gradually liquefied, the grayish white growth masses settling to the bottom of the colony. Under the microscope, the colony presents the usual granular appearance, the edge of it being sharply defined. In gelatin tubes, the appearance of the cultures is more characteristic. It spreads superficially more rapidly than in vertical direction, forming a shallow cup filled with homogeneously granular, turbid, liquefied gelatin that is covered with a copious white film on surface. The predilection for oxygen is further shown when cultures are made under anaerobic conditions. In deep gelatin tubes and in cultures prepared according to Buchner's pyrogallic method, it does not develop.

The liquefaction of the gelatin in ordinary tube cultures progresses slowly until the side of the tube is reached and then continues steadily to the bottom in horizontal layers. In old cultures, the film on the surface often has a reddish hue. The appearance along submerged portions of the needle track indicates only a scant growth.

Streak cultures on agar show a thin hazy film becoming more opaque in lower part of tube where condensation water collects. On agar plate cultures, considerable variation is to be noted. Sometimes the colonies are small and opaque, then again they are often spread out in a thin film as in tubes. The colonies on agar are more or less viscid, and adhere easily to the needle when touched.

On potato, this bacillus is a chromogene and grows more luxuriantly on this than any other medium. In thirty-six hours, at room temperature, the culture growth is apparent where it appears as a glistening yellowish drop around inoculation puncture.

This rapidly spreads until the whole surface of potato is often covered with a deep orange-yellow growth that changes later in old cultures to a brick red.

Milk is modified profoundly, the casein being coagulated by the production of acids. This coagulated material is soon peptonized, the serum remaining more or less cloudy.

Bacillus litorosus, sp. nov.—This form was found in both water and mud layers in not inconsiderable numbers, although not nearly so common as B. pelagicus.

Morphologically, it belongs to the larger type of bacteria, being a slender, typical bacillus form ($1\mu \times 4$–7μ). It is usually found as an isolated individual or closely united in short chains of two or four segments. The protoplasm of the cells is usually homogeneous. This form is motile and is characterized by a slow, stately movement of a serpentine nature.

In ordinary gelatin media, it forms quite a characteristic culture. Two or three days after inoculation, liquefaction of gelatin is to be noted at the surface and along needle track as well. The peptonizing of this substance takes place more rapidly at surface, so a broad funnel shaped sac is formed as a result of two or three days' growth. There is usually a sharply defined air pit at the surface, the base of which is covered with grayish masses of zooglœa. In the lower part of the liquefied needle track, spirally twisted strands are to be noted. A thick membrane is finally formed on surface from bits of flocculent zooglœa that adhere in masses which easily rupture upon the slightest movement. In old cultures, the bottom of the liquefied gelatin is covered with flocculent growth masses and the culture is not especially characteristic.

On agar, this bacillus grows rapidly, forming a thick dull white colony with a sharply defined border. Under the lens, the edge of the colony is seen to be formed of numerous parallel filamentous bands, that are looped at the edge so there are no freely projecting filaments radiating into the surrounding medium.

In agar tubes, these well defined grayish patches become confluent and more luxuriant in the lower part of the tube. At room temperature, the germ thrives better than at blood heat. In deep stick gelatin or agar cultures as in bouillon tubes prepared according to Buchner's pyrogallic acid method, a marked growth is to be noted, showing the facultative nature of the germ.

On potato there is a well defined compact white growth that is soft in texture and easily detached. When inoculated into milk, this bacillus sets up a very rapid change coagulating the casein and finally peptonizing it. The serum of the milk is at first filled with a cloudy precipitate that is formed from the masses of zoogloea but these are finally deposited on the coagulated casein.

Bacillus maritimus, sp. nov.—Bacillus maritimus is found quite plentifully distributed in the mud layers. It is a sporogenous bacillus of good size (dimensions $1.50\mu \times 3.5$–6μ) with round ends, but the cells are usually quite closely united into filaments of varying length. The protoplasm has often a granular appearance so characteristic of marine bacterial forms. The spores are small, scarcely exceeding one-half the width of the cell ($0.7\mu \times 1.5\mu$). These are readily formed in forty-eight hours at the room temperature.

On artificial media this germ grows luxuriantly. It is seemingly indifferent to the salt content of the substratum upon which it develops. In gelatin, two days growth is marked as follows: a very shallow pit spreads out over surface, the bottom of which is covered with a grayish growth that has the peculiar luster so characteristic of the freshly broken surface of limestone. From the base of this shallow depression fine filaments radiate for a short distance into the gelatin. Growth along needle track is barely visible, thus showing the aerobic nature of this germ. In three days, this superficial growth spreads slowly to the sides of the tube and the gelatin is slowly peptonized in horizontal layers, the liquefied material being filled with cloudy masses. An imperfect film that is easily ruptured is often to be observed on surface of culture.

On agar, growth is copious but not especially characteristic. The surface of the medium is covered with a dense white covering smooth in character.

On potato it also thrives luxuriantly as a thick grayish white coating covering nearly whole surface of potato. Surface of growth is dull and mealy in texture.

Conclusions.

It may be well to summarize briefly the results that have been reached in the foregoing pages in the following general conclusions:

1. Bacteria are present in the oceanic waters, both of the high seas and in litoral regions, although they are not so abundant as they are in fresh water masses.

2. While the bacterial flora of the sea bottom in the region investigated this year is not near so great, from a numerical standpoint, as that in the neighborhood of Naples, yet the accuracy of the general conclusions drawn from the Mediterranean work in regard to the slime bacteria is strengthened by the present season's work.

Bacteria exist in the mud of the ocean bottom in large numbers, and their presence there is not due merely to the effect of gravity but to the growth and reproduction of indigenous slime species. Forms that are prevalent in the water masses above are also found in the mud layers in a vegetating as well as in a latent stage of development.

3. Disregarding the influence of introduced forms from the land, which is in this case very slight, the indigenous marine flora of both water and sea floor is largely comprised within the limits of relatively few species.

These may often be distributed over more than localized areas, and in some instances come to be cosmopolitan in their range. Not only is their geographical distribution often extensive but the vertical range of the various forms exceeds that of the majority of the higher forms of life.

4. While no pathogenic property was noted in any of the forms isolated, other physiological characters, as the formation of soluble enzymes and the ability to reduce nitrate salts, were conspicuous characteristics of the more prevalent forms.

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EXPLANATION OF PLATE XXXVI.—Figures of bacteria in cultures drawn with a Zeiss microscope, tube length 160^{mm}, no. 6 compensating ocular and $\frac{1}{12}$ homogeneous oil immersion.

Fig. 1. *A. Bacillus limicola*, gelatin culture 5 days old.

Fig. 1. *B. a*, Short type of cells from agar culture; *b*, lanceolate type from old agar culture; *c*, normal bacillus type from agar culture, 3 days old.

Fig. 2. *A. Bacillus pelagicus*, culture in gelatin.

Fig. 2. *B.* Spore bearing bacilli from gelatin culture, two weeks old.

Fig. 3. *A. Bacillus maritimus*, gelatin culture, 2 days old.

Fig. 3. *B.* Single cells from potato culture, 2 days old.

Fig. 4. *A. Bacillus litorosus*, 3 days growth in gelatin.

Fig. 4. *B.* Cells from young gelatin culture.

Studies in the biology of the Uredineæ. I.

Notes on germination.

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WITH PLATES XXXVII–XXXIX.

Within the last eighteen months I have made about four hundred cultures of uredineal spores, in various media, at various seasons, in order (1) to determine as many facts as possible concerning their biology on the one hand, and on the other hand, by observing the various effects of different chemicals upon their germination, (2) to obtain a working basis for the preparation of fungicides for the prevention of the attacks of certain economic species. A great part of the work, particularly that of most economic importance, has already been reported.[1] What I shall present here, are some results and suggestions of a more technical nature.

Nearly all the cultures made were of the ordinary drop culture style, prepared by depending a drop of the employed medium, in which the spores were immersed, from the lower surface of a cover-glass placed over an ordinary glass cell

[1] Kansas Agric. Exp. Sta. Bull. *38*. Mar. 1893.